EFFECTS OF FLAXSEED (*LINUM USITATISSIMUM*) CHUTNEY ON GAMMA-GLUTAMYL TRANSPEPTIDASE AND MICRONUCLEI PROFILE IN AZOXYMETHANE TREATED RATS

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ABSTRACT

Antioxidant property of flaxseed chutney was evident by decreasing lipid peroxidation (TBARS) and predictor enzyme γ -glutamyl transpeptidase profile and micronuclei formation in azoxymethane treated rats. After 10 weeks, rats fed with either fiber-free basal diet or Antioxidant diet exhibited over sevenfold increase in γ -glutamyl transpeptidase activity and nearly fourfold increase in micronuclei load in comparison to controls ($p < 0.001$). A significant reduction in both γ -glutamyl transpeptidase level (52%) and micronuclei formation (47%) was observed in fiber-free basal diet / Antioxidant diet / flaxseed chutney diet fed rats. Relative to rats fed fiber-free basal diet, the profile of γ -glutamyl transpeptidase and micronuclei load was not significantly altered.

KEY WORDS

Flaxseed chutney, Micronuclei, γ -glutamyl transpeptidase profile, Azoxymethane, TBARS

INTRODUCTION

Flaxseed has long history of use in India. Flaxseed preparations are particularly considered for its nutrients and therapeutic property (1). In southern India, flaxseed is partly being consumed at lower levels as flaxseed *chutney* (FC). *Linum usitatissimum* L is commonly known as linseed or flaxseed belongs to the family *Linoceae*. Flaxseed is exceptionally a rich source of mammalian lignan precursor secoisolariciresinol diglucoside (SDG) present at levels 75 – 800 times greater than any other plant known to this date (2). Scientists at American National Cancer Institute singled out flaxseed as one of six nutraceuticals for food applications (3).

Microsomal enzyme γ -glutamyl transpeptidase (γ -GT) has been reported to be a marker of pre-neoplastic lesions and richly present in liver and kidney. γ -GT levels are increased abnormally high during inflammation, in alcoholics with liver

abscess and degenerative disease conditions including tumorigenesis (4). There are numbers of studies indicating the role of raw flaxseed and its baked products in health promotion and disease prevention (5,6). Preliminary studies on FC in rats from our laboratory showed encouraging results on lipid lowering action and protection against liver damage (7). However, systematic studies on health benefits of flaxseed *chutney* have not been worked out so far. This stimulated us to make an attempt to assess effect of flaxseed *chutney* on γ -GT marker enzyme profile and micronuclei formation in azoxymethane-induced rats.

MATERIALS AND METHODS

Seed material and flaxseed *chutney* preparation : *Linum usitatissimum* seeds (brown variety) were procured from the local market of Davangere, Karnataka State, India, during April-May 2001. The University of Agricultural Sciences, Hebbal, Bangalore, India identified the seeds. A specimen sample of seeds (LVF01) was preserved for reference.

The traditional method (of preparing flaxseed-based *chutney* practiced in Southern India) was used to produce the FC diet. The FC was mainly composed of flaxseed (0.78g/kg), tamarind (0.02g/kg), jiggery (0.02g/kg) and garlic (0.060 g/kg). The ingredients were added with chili powder (0.04 g/kg) after a brief roasting (5-min). The materials were pounded to a fine

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powder (80-100 mesh) by adding little salt (0.04g/kg). Separately roasted and powdered cumin seeds (0.004g/kg) were admixed and pounded further to a fine powder (100-120 mesh), transferred to a jar, covered with a lid and stored at room temperature until use.

Animals and feeding design : The laboratory animals used for the present study were mature male Wister rats weighing between 95-105 g, maintained in the Department of Small Animals, Veterinary College, Hebbal, Bangalore, India. Rats were divided into four groups of six animals each. All the rats were housed in polypropylene cages with stainless steel grill top bedded with rice husk. Animals were maintained under standard conditions (22-25°C; 60-70% relative humidity; 12-h dark/day cycle) fed normal and test diet and water *ad libitum*. The animals were sacrificed according to the guidelines of the current laws of CPCSEA (Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals), India.

Relative composition of two experimental diets is given Table 1. Group A- served as control, basal diet (BD) consisting of casein, dl- methionine, groundnut vegetable oil, mineral mix, vitamin mix and cornstarch. Group B- animals fed basal diet supplemented with 15% FC. Group C- animals fed basal diet and toxicant azoximethane (15mg/kg b.w, i.p; 10 weekly injections). Group D- animals fed FC supplemented diet and toxicant azoximethane (15mg/kg b.w, i.p; 10 weekly injections).

After 10 weeks, the mature rats were sacrificed under mild anesthesia. The liver, kidney and femur were immediately excised and placed in pre-chilled 0.25 M sucrose solution, and stored below 0°C until further use. The tissue sample from all the killed animals was homogenized to form 5% homogenate of enzyme extract. The homogenates of tissues prepared in sucrose solution and were then centrifuged for 15 minutes at 12000 rpm in a cooling centrifuge and clear supernatants were used as a source of enzyme. The enzyme extracts were kept at 0°C until required.

Inhibition of liver γ -GT activity and liver lipid peroxide formation: For the determination of γ -glutamyl transpeptidase (γ -GT) activity in kidney Meister *et al* method was employed (8). L- γ -glutamyl-p-nitroanilide as substrate in a homogenate prepared with 0.1M Tris-HCl buffers. Lipid peroxide content in the liver was determined as thiobarbituric acid reactive substances (TBARS) using method of Ohkawa *et al.* (9).

Inhibition of bone marrow micronuclei formation: For micronuclei determination, bone marrow from the femur was

flushed out using ice-cold phosphate buffer saline. The contents were centrifuged (at 10 °C, 1000 rpm for 10 min). The pellet was suspended in 5% bovine serum albumin solution for 5 min and then centrifuged (x 2). The smears prepared on slides were air-dried, methanol-fixed and stained with Giemsa (2%). Micronuclei were counted as described by Countryman and Heddle (10). On an average 6000 cells were counted and frequency of cells with micronuclei formation (Mf) was accounted (%) as: $Mf = \frac{Nm}{Nt} \times 100$, Where 'Nm' is the number of cells with micronuclei and 'Nt' is the total number of cells counted. All the experiments were repeated five times and the data were subjected to statistical analysis for 't'-test to know the level of significance.

RESULTS AND DISCUSSION

Liver γ -GT profile and liver lipid peroxidation : The experimental diets used in this study and their composition is given in Table 1. Long-term exposure to azoximethane caused adverse effects in liver of mature rats. The damage was most significant as observed in the activity of γ -glutamyl transpeptidase, which increased over sevenfold among group C rats (5.97 μ mol/mg protein) upon treatment with azoximethane versus control (0.83 μ mol/mg protein) (Table 2). In FC supplemented rats (group D), γ -glutamyl transpeptidase profile was significantly decreased to 3.11 μ

Table 1 : Composition of the two experimental diets

Ingredients	Basal diet (g/kg)	FC diet (g/kg)
Flaxseed <i>chutney</i>	—	150
Pregelatinized cornstarch*	650	500
Casein	240	240
Lard	58	58
DL-Methionine	4	4
Refined groundnut oil	18	18
Minerals ¹	44	44
Vitamins ²	2	2

*Cerestar SF 12018, Belgium

¹Mineral composition (mg/kg diet): CaHPO₄ · 2H₂O, 16720.00; K₂HPO₄, 10560.00; CaCO₃, 8028.24; NaCl, 3080.00; MgO, 880.00; MgSO₄ · 7H₂O, 3960.00; FeSO₄ · 7H₂O, 308.00; ZnSO₄ · H₂O, 220.00; MnSO₄ · H₂O, 198.00; CuSO₄ · 5H₂O, 44.00; CoCO₃, 0.44; KI, 0.44; (NH₄)₆ Mo₇O₂₄ · 4H₂O, 0.44; NaSeO₃, 0.44.

²Vitamin composition (mg/kg diet): retinal, 16.20; thiamin, 2.00; riboflavin, 3.20; nicotinic acid, 16.20; calcium pantothenate, 16.20; pyridoxine, 2.00; inositol, 40.40; biotin, 8.10; folic acid, 0.80; vitamin B-12, 24.20; ascorbic acid, 40.40; cholecalciferol, 1.60; vitamin E, 10.71; menodione, 1.60; para-aminobenzoic acid, 40.40; choline, 502.54;

mol/mg protein) ($p < 0.002$) measuring about 52% in comparison to group C rats. Marginal increase on γ -glutamyl transpeptidase activity ($1.10 \mu\text{mol/mg protein}$) was observed among group B versus control rats ($0.83 \mu\text{mol/mg protein}$). The TBARS generated at physiological pH in control rats (21.30 Units/L) and AOM plus BD treated rats (24.50 Units/L) was inhibited by 15% FC supplemented diet, in the order more than 29% and 33%, respectively.

Bone marrow micronuclei count - The micronuclei test, conducted on the bone marrow cells is considered to be a highly reliable test of precancerous changes in tissues (11), which is supported by WHO (1990). The Mf (%) value of rats treated with azoximethane was remarkably increased. In-group D rats fed 15% FC supplemented diet the number of cells with micronuclei fraction was significantly decreased ($>47\%$) when compared to group C rats. Group B rats fed alone 15% FC supplemented diet did show further decrease in Mf (%) values than its control group (Table 2).

Table 2 : Effect of feeding flaxseed (*Linum usitatissimum*) chutney on the profile of liver γ -glutamyl transpeptidase activity, liver lipid peroxidation and bone marrow micronuclei formation in mature male Wister rats

Treatment Groups	γ -GT activity ($\mu\text{mol/mg protein}$)	TBARS (Units/L)	Micronuclei frequency (%)
Group A (BD)	0.83 ± 0.04	21.30 ± 0.05	0.433 ± 0.004
Group B (BD + 15% FC)	1.10 ± 0.04	15.10 ± 0.01	0.266 ± 0.190
Group C (BD + AOM)	5.97 ± 0.37	24.50 ± 0.34	1.583 ± 0.260
Group D (BD + AOM + 15% FC)	3.11 ± 0.19	16.30 ± 1.08	0.750 ± 0.100

Values are mean $\pm$ SD (n=6); Student's t-test. $P < 0.05$. AOM (15mg/kg b.w, i.p; 10 weekly injections) was administered to group C and D. γ -GT activity: micromoles of p-nitroanilide released/min/g kidney.

This practical intervention study reveals protective effect of flaxseed chutney, which is having a great capacity for prevention of liver damage by exerting hepatoprotective activities against azoximethane. The traditional FC supplemented diet could significantly reduce liver lipid peroxidation; liver γ -GT profile and micronuclei formation in rats treated with AOM and thus protects the mature rats from toxicant. The antioxidant property of FC may be due to high amount of lignan SDG from flaxseed (12). Under this scenario, it necessitates the generation of comprehensive data on

potential therapeutic non-nutrient principles present in the flaxseed chutney and its clinical relevance, and the authors are continuing this.

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